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(FILE 'MEDLINE, HCPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
13:24:17 ON 12 MAR 2003)

L26 33 DUP REM L25 (21 DUPLICATES REMOVED)

=> d que 126

L1 622 SEA COELHO P?/AU
L2 410 SEA KINGSLEY P?/AU
L3 11 SEA BRAUSCH J?/AU
L4 79 SEA GODSEY J?/AU
L5 1394 SEA ROCK G?/AU
L6 2500 SEA (L1 OR L2 OR L3 OR L4 OR L5)
L7 32 SEA L6 AND THROMBIN#
L8 9 SEA L7 AND (STABLE OR STABIL?)
L9 1 SEA L7 AND ENRICH?
L10 4 SEA L7 AND PURIF?
L11 4798 SEA PLASMA(5A) CENTRIFUG?
L12 160 SEA L11 AND THROMBIN#
L13 10 SEA L12 AND (ETHANOL OR ETHYL(A) ALCOHOL)
L14 3 SEA THROMBIN# (5A) (PURIF? OR ENRICH? OR ISOLAT?) (5A) (ETHANOL
OR ETHYL(A) ALCOHOL)
L15 66 SEA THROMBIN# (5A) (PURIF? OR ENRICH? OR ISOLAT?) (5A) (CALCIUM
OR CA OR CACL2)
L16 11 SEA L15 AND PROTHROMBIN#
L17 2 SEA L15 AND THREONE
L19 15 SEA L15 (5A) ACTIVAT?
L20 14 SEA L14 OR L16 OR L17
L21 23 SEA L20 OR L19
L22 7 SEA THROMBIN# (5A) (ETHANOL OR ETHYL(A) ALCOHOL) (5A) (CALCIUM
OR CA OR CACL2)
L23 30 SEA PLASMA AND THROMBIN# AND (ETHANOL OR ETHYL(A) ALCOHOL) AND
(CALCIUM OR CA OR CACL2)
L24 3 SEA L23 AND FILT?
L25 54 SEA L8 OR L9 OR L10 OR L13 OR L21 OR L22 OR L24
L26 33 DUP REM L25 (21 DUPLICATES REMOVED)

=> d ibib abs 126 1-33

L26 ANSWER 1 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2003:14680 BIOSIS

DOCUMENT NUMBER: PREV200300014680

TITLE: Method for preparing **thrombin** for use in a
biological glue.

AUTHOR(S): Coelho, Philip Henry; Kingsley, Phil;
Brausch, Jim; Godsey, James H.;

Rock, Gail (1); Madsen, Trista K.; Frausto, Sona B.

CORPORATE SOURCE: (1) Ottawa, Canada Canada

ASSIGNEE: ThermoGenesis Corp., Rancho Cordovo, CA, USA

PATENT INFORMATION: US 6472162 October 29, 2002

SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (Oct. 29 2002) Vol. 1263, No. 5, pp. No
Pagination. <http://www.uspto.gov/web/menu/patdata.html>.
e-file.

ISSN: 0098-1133.

DOCUMENT TYPE: Patent

LANGUAGE: English

AB A sterile method for preparing **stable thrombin**

component from a single donor's plasma in which the **thrombin** component and the clotting and adhesive proteins component are harvested simultaneously from the same donor plasma in less than one hour. The combined components provide an improved biological hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The **thrombin** provides polymerization of the clotting and adhesive proteins in less than five seconds, and is sufficiently **stable** to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by diluting the **thrombin** with saline.

L26 ANSWER 2 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:465846 BIOSIS
DOCUMENT NUMBER: PREV200100465846
TITLE: Apparatus and method of preparation of **stable**,
long term **thrombin** from plasma and
thrombin formed thereby.
AUTHOR(S): Coelho, Philip Henry; Kingsley, Phil (1)
; Brausch, Jim; Godsey, James H.;
Rock, Gail
CORPORATE SOURCE: (1) Sacramento, CA USA
ASSIGNEE: ThermoGenesis Corp.
PATENT INFORMATION: US 6274090 August 14, 2001
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (Aug. 14, 2001) Vol. 1249, No. 2, pp. No
Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB A sterile method for preparing **stable thrombin**
component from a single donor's plasma in which the **thrombin** component is harvested simultaneously from the clotting and adhesive proteins component from the same donor plasma in less than one hour. The combined components provide an improved biological hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The **thrombin** provides polymerization of the clotting and adhesive proteins in less than five seconds, and is sufficiently **stable** to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by diluting the **thrombin** with saline.

L26 ANSWER 3 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:880991 HCPLUS
DOCUMENT NUMBER: 134:32945
TITLE: Preparation of autologous **thrombin** from
blood plasma
INVENTOR(S): Coelho, Philip H.; Kingsley, Phil;
Brausch, Jim; Godsey, James H.;
Rock, Gail; Madsen, Trista K.; Frausto, Sona
B.
PATENT ASSIGNEE(S): Thermogenesis Corp., USA
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000074713	A1	20001214	WO 2000-US11865	20000602
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
US 6472162	B1	20021029	US 1999-328350	19990604
EP 1198242	A1	20020424	EP 2000-939285	20000602
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL			
PRIORITY APPLN. INFO.:			US 1999-328350	A 19990604
			WO 2000-US11865	W 20000602

AB A sterile method for prep. **stable thrombin** component from a single donor's plasma in which the **thrombin** component and the clotting and adhesive proteins component are harvested simultaneously from the same donor plasma in <1 h. The combined components provide an improved biol. hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The **thrombin** provides polymn. of the clotting and adhesive proteins in <5 s, and is sufficiently **stable** to provide that fast clotting over a 6-h period. Further, the clotting times can be predictably lengthened by dilg. the **thrombin** with saline. Thus, a compn. for extg. **thrombin** from plasma consists of plasma, 18.9% EtOH, 23.0 mM CaCl₂, and glass beads. A method for generating and then dispensing **thrombin**, included the steps; taking whole blood from one person, sequestering prothrombin from the whole blood, converting the prothrombin to **thrombin**, loading the **thrombin** into a syringe, and and then applying the **thrombin** onto an area to stem blood flow.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 4 OF 33 HCPLUS COPYRIGHT 2003 ACS
 ACCESSION NUMBER: 2000:116937 HCPLUS
 DOCUMENT NUMBER: 132:156811
 TITLE: Apparatus and method of preparation of **stable**, long term autologous **thrombin** from plasma and **thrombin** formed thereby
 INVENTOR(S): Coelho, Philip Henry; Godsey, James H.; Brausch, Jim; Kingsley, Phil; Rock, Gail
 PATENT ASSIGNEE(S): Thermogenesis Corp., USA
 SOURCE: PCT Int. Appl., 31 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000007659	A1	20000217	WO 1999-US16698	19990805

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6274090 B1 20010814 US 1998-129988 19980805

AU 9954591 A1 20000228 AU 1999-54591 19990805

EP 1104323 A1 20010606 EP 1999-940810 19990805

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: US 1998-129988 A 19980805
WO 1999-US16698 W 19990805

AB A sterile method is disclosed for prepg. **stable thrombin** component from a single donor's **plasma** in which the **thrombin** component is harvested simultaneously from the clotting and adhesive proteins component from the same donor **plasma** in less than one hour. The combined components provide an improved biol. hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The **thrombin** provides polymn. of the clotting and adhesive proteins in less than five seconds, and is sufficiently **stable** to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by dilg. the **thrombin** with saline.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 5 OF 33	MEDLINE	DUPPLICATE 1
ACCESSION NUMBER:	1999071879 MEDLINE	
DOCUMENT NUMBER:	99071879 PubMed ID: 9854715	
TITLE:	Rapid preparation of small-volume autologous fibrinogen concentrate and its same day use in bleb leaks after glaucoma filtration surgery.	
AUTHOR:	Gammon R R; Prum B E Jr; Avery N; Mintz P D	
CORPORATE SOURCE:	Blood Bank and Transfusion Services, University of Virginia, Charlottesville 22908, USA.	
SOURCE:	OPHTHALMIC SURGERY AND LASERS, (1998 Dec) 29 (12) 1010-2. Journal code: 9517132. ISSN: 1082-3069.	
PUB. COUNTRY:	United States	
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)	
LANGUAGE:	English	
FILE SEGMENT:	Priority Journals	
ENTRY MONTH:	199903	
ENTRY DATE:	Entered STN: 19990324 Last Updated on STN: 20000303 Entered Medline: 19990310	

AB The authors evaluated small-volume preparation of autologous fibrin glue (AFG) and same day use in postglaucoma filtration surgery patients with Seidel positive bleb leaks and determined fibrinogen concentrations in autologous fibrinogen concentrates (AFCs) from 10 volunteers. Thirty milliliters of blood was **centrifuged** (5 min, 2400 x g); **plasma** was frozen (5 min-**ethanol** and ice), thawed (1-6 C, 30-60 min), and centrifuged (10 min, 5 C, 2800 x g); and the precipitate was transferred to a 1.0-ml tuberculosis syringe. **Thrombin** (1000 U) was dissolved (0.8 sterile water, 0.2 ml

aminocaproic acid) and warmed (37 C). Average preparation time was 90 minutes. Alternating drops of AFC and **thrombin** were applied to bleb leaks until AFC clotted. Seidel testing with fluorescein determined success. AFC was prepared from 10 volunteers and fibrinogen was measured. AFG was initially successful with two (Seidel negative) eyes; one eye remained negative. AFG was unsuccessful in one briskly Seidel-positive leak. Mean +/- SD fibrinogen concentration in AFCs from the 10 volunteers was 2314 +/- 643 mg/dl (range 1608-3431 mg/dl). AFG may successfully close bleb leaks in outpatient settings. Brisk aqueous flow may impair effectiveness of AFG. Fibrinogen concentrations were comparable with previous reports.

L26 ANSWER 6 OF 33 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 95053337 MEDLINE
 DOCUMENT NUMBER: 95053337 PubMed ID: 7964131
 TITLE: Changes of cytosolic calcium ion concentrations in human endothelial cells in response to thrombin, platelet-activating factor, and leukotriene B4.
 AUTHOR: Lerner R
 CORPORATE SOURCE: Department of Medicine, Karolinska Institute, Stockholm Soder Hospital, Sweden.
 SOURCE: JOURNAL OF LABORATORY AND CLINICAL MEDICINE, (1994 Nov) 124 (5) 723-9.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
 ENTRY MONTH: 199412
 ENTRY DATE: Entered STN: 19950110
 Last Updated on STN: 19950110
 Entered Medline: 19941221

AB This study concerns changes of intracellular calcium concentrations, [Ca²⁺]_i, in human umbilical vein endothelial cells (HUVECs) in response to thrombin, platelet activating factor (PAF), and leukotriene B4 (LTB4). Thrombin (0.003 to 1 U/ml) induced a dose-dependent, pertussis toxin-insensitive rise of [Ca²⁺]_i that was initially rapid and transient and was followed by a sustained plateau phase that required extracellular calcium to be expressed. Early during that plateau a second rise of [Ca²⁺]_i was seen that was amplified in the presence of propranolol and abolished in calcium-free medium or by ethanol. Repeated stimulations with **thrombin** evoked renewed but declining responses. Pretreatment of HUVECs with PAF or lipoxin A4, but not LTB4, diminished a subsequent response to thrombin. PAF induced a small, dose-dependent increase of [Ca²⁺]_i with kinetics similar to that of thrombin. It was blocked by previous exposure of HUVECs to PAF and thrombin but not to LTB4 or pertussis toxin. LTB4 induced a rapid but sustained increase of [Ca²⁺]_i that resembled that of the calcium ionophore ionomycin. In contrast to responses to thrombin and PAF, it could be abolished by previous treatment with pertussis toxin and required extracellular calcium. Addition of LTB4 after previous stimulation with LTB4 or PAF gave no detectable response. Implications of these results for some specific signal transduction mechanisms for agonists studied are discussed.

L26 ANSWER 7 OF 33 MEDLINE DUPLICATE 3
 ACCESSION NUMBER: 95207979 MEDLINE
 DOCUMENT NUMBER: 95207979 PubMed ID: 7900071
 TITLE: Demonstration of platelet-derived microvesicles in blood

from patients with activated coagulation and fibrinolysis using a filtration technique and western blotting.
 AUTHOR: Holme P A; Solum N O; Brosstad F; Roger M; Abdelnoor M
 CORPORATE SOURCE: Research Institute for Internal Medicine, Rikshospitalet, Norway.
 SOURCE: THROMBOSIS AND HAEMOSTASIS, (1994 Nov) 72 (5) 666-71.
 PUB. COUNTRY: Journal code: 7608063. ISSN: 0340-6245.
 DOCUMENT TYPE: GERMANY: Germany, Federal Republic of
 LANGUAGE: Journal; Article; (JOURNAL ARTICLE)
 English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199504
 ENTRY DATE: Entered STN: 19950504
 Last Updated on STN: 19950504
 Entered Medline: 19950426

AB Platelet vesiculation in vitro is correlated to platelet activation. It was therefore considered of interest to see if microvesicles (MV) are present in blood in clinical situations associated with platelet activation in vivo. Patients with both activated coagulation and fibrinolysis, implying that **thrombin** has been generated, suit such a purpose. Thus, the aim of this study was to investigate whether microvesicles could be detected in patients with activated coagulation and fibrinolysis, as diagnosed by the presence of soluble fibrin (positive **ethanol** gelation tests) and positive tests for fibrin degradation products (FDP). Platelet-rich plasma was prepared from citrated blood from patients (n = 22) and healthy controls (n = 32) matched as to age and sex. The intact platelets were removed from **plasma** by **centrifugation**. Any MV present were isolated from the platelet-free plasma by a filtration procedure, washed, solubilized in Triton X-100 and subjected to SDS-PAGE with Western blotting using a MAb against GPIIb alpha as an indicator of the presence of microvesicles. All of the 22 patients showed the presence of microvesicles detectable by the content of GPIIb alpha, whereas this could be observed in only 4 out of the 32 normal controls and then in small or trace amounts only. The presence of microvesicles among cell-derived material in the plasma of two of the patients was also confirmed by electron microscopy. To the best of our knowledge this is the first report on the presence of microvesicles in plasma from patients with both activated coagulation and fibrinolysis. (ABSTRACT TRUNCATED AT 250 WORDS)

L26 ANSWER 8 OF 33 HCPLUS COPYRIGHT 2003 ACS
 ACCESSION NUMBER: 1994:100555 HCPLUS
 DOCUMENT NUMBER: 120:100555
 TITLE: Preparation of thrombin from animal blood
 INVENTOR(S): Liu, Gaodong; Huang, Weidong
 PATENT ASSIGNEE(S): Peop. Rep. China
 SOURCE: Faming Zhanli Shengqing Gongkai Shuomingshu, 4 pp.
 CODEN: CNXXEV
 DOCUMENT TYPE: Patent
 LANGUAGE: Chinese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1074709	A	19930728	CN 1992-112188	19921126
PRIORITY APPLN. INFO.:			CN 1992-112188	19921126

AB A cost-effective method for prepn. of thrombin from animal blood is described. The blood sample mixed with anticoagulant, EtOH, and a

buffering agent is centrifuged to obtain a ppt. contg. prothrombin (at pH 5.3). The ppt. is solubilized in water, chromatographed through a heparin-agarose column, and the eluate is further pptsd. at pH 4.4 to obtain purified prothrombin. Prothrombin is then activated by incubation at room temp. for >1 h and thrombin purified by chromatog. on the G-25 column at pH 7.2 in the presence of CaCl₂.

L26 ANSWER 9 OF 33 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 93:722939 SCISEARCH
THE GENUINE ARTICLE: MJ657
TITLE: CHARACTERIZATION OF FACTORS AFFECTING THE
STABILITY OF FROZEN HEPARINIZED PLASMA
AUTHOR: PALMER D S (Reprint); ROSBOROUGH D; PERKINS H; BOLTON T;
ROCK G; GANZ P R
CORPORATE SOURCE: CANADIAN RED CROSS SOC, OTTAWA CTR, 85 PLYMOUTH ST, OTTAWA
K15 3E2, ON, CANADA (Reprint); UNIV OTTAWA, FAC MED, DEPT
MED, OTTAWA K1N 6N5, ONTARIO, CANADA; UNIV OTTAWA, FAC
MED, DEPT BIOCHEM, OTTAWA K1N 6N5, ONTARIO, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: VOX SANGUINIS, (1993) Vol. 65, No. 4, pp. 258-270.
ISSN: 0042-9007.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 60

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The use of heparin rather than citrate as primary anticoagulant has been shown to significantly improve the initial activity, stability and recovery of factor VIII:C from human plasma, cryoprecipitates or factor VIII concentrates if the plasma was initially frozen at -80-degrees-C and subsequently stored at this temperature. If frozen and stored at progressively warmer temperatures however, increasing amounts of insoluble protein aggregates, termed storage precipitates (SPs), were recovered in the thawed plasma and cryoprecipitate fractions. Plasma recovery by centrifugation at 7,000 g for 7 min [Method I (MI)], 2 X 10 min (MII) or 15 min (MIII) had little effect on SP formation after 1 month at any storage temperature. After 4 months at -20-degrees-C, more SP was recovered from MIII plasma whereas at -40-degrees-C, more SP was recovered from MI plasma. Also, the preparation method had little or no effect on factor VIII:C activity at equivalent storage times or temperatures. A trend towards improved factor VIII recoveries was noted at lower freezing and storage temperatures however. SP formation was associated with reduced fibrinogen levels in the recovered plasma without loss of antithrombin-III or increased fibrinopeptide-A. Western blots showed polymerization of Aalpha or gamma-chains of fibrinogen. SP formation was reduced or eliminated with factor XIII inhibitors, antibody to the active factor XIII a subunit or adjustment of heparinized plasma to 5-10 mM sodium citrate before initial freezing and storage. Although plasma factor VIII:C recoveries were only slightly affected at these citrate concentrations under most conditions, its recovery in cryoprecipitates was substantially improved owing to the reduction or absence of SPs.

L26 ANSWER 10 OF 33 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 93208257 MEDLINE
DOCUMENT NUMBER: 93208257 PubMed ID: 8384497
TITLE: Haemostatic derangements associated with arenavirus infection in the guinea-pig: radioimmunoassay of fibrinopeptide A to assess thrombin action in

infected animals.
AUTHOR: Chen J P; Marsh L C; Schroeder E C
CORPORATE SOURCE: Department of Medical Biology, University of Tennessee
Medical Center/Knoxville 37920.
SOURCE: BLOOD COAGULATION AND FIBRINOLYSIS, (1993 Feb) 4 (1)
165-72.
PUB. COUNTRY: Journal code: 9102551. ISSN: 0957-5235.
ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930514
Last Updated on STN: 19980206
Entered Medline: 19930427
AB Pichinde virus infection of inbred guinea-pigs is a model for arenaviral infections in humans. Infected animals experience reduced levels of multiple coagulation factors caused by either consumption coagulopathy or impaired factor synthesis. A radioimmunoassay (RIA) of guinea-pig fibrinopeptide A (gFPA) has been developed to measure the degree of **thrombin** action in vivo. gFPA was synthesized via the solid-phase method and conjugated to bovine serum albumin (BSA). A double antibody RIA was established employing goat anti-rabbit IgG to precipitate the primary complex composed of either 125I-5-Tyr-gFPA or 125I-12-Tyr-gFPA and rabbit anti-gFPA-BSA. The cross-reacting material was removed by mixing the plasma with 3 vol of **ethanol**. The supernatant was filtered through a hollow fibre apparatus by **centrifugation**.
Plasma gFPA immunoreactivities of outbred guinea-pigs averaged 6.56 ng/ml. The gFPA-RIA was validated by determining the quantity of gFPA released from **thrombin**-degraded fibrinogen. A transient elevation of gFPA levels was detected in Pichinde-infected animals by the gFPA-RIA using 125I-12-Tyr-gFPA as a tracer. The pathogenic mechanism by which the increased gFPA levels may lead to the lethality of Pichinde virus infection remains to be elucidated. It is possible that the coagulopathy triggers changes in immune and inflammatory pathways that induces high cytokine concentrations, with deleterious effects on organs such as the heart and lungs.

L26 ANSWER 11 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:493199 HCPLUS
DOCUMENT NUMBER: 113:93199
TITLE: Ethanol inhibits thrombin-induced secretion by human platelets at a site distinct from phospholipase C or protein kinase C
AUTHOR(S): Benistant, Christine; Rubin, Raphael
CORPORATE SOURCE: Dep. Pathol. Cell Biol., Jefferson Med. Coll., Philadelphia, PA, 19107, USA
SOURCE: Biochemical Journal (1990), 269(2), 489-97
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addn. of physiol. relevant concns. of ethanol (25-150 mM) to suspensions of washed human platelets resulted in the inhibition of thrombin-induced secretion of 5-hydroxy[14C]tryptamine. Indomethacin was included in the incubation buffer to prevent feedback amplification by arachidonic acid metabolites. Ethanol had no effect on the activation of phospholipase C by thrombin, as detd. by the formation of inositol phosphates and the mobilization of intracellular Ca²⁺. Ethanol did not interfere with the thrombin-induced formation of diacylglycerol or

phosphatidic acid. Stimulation of platelets with phorbol ester (5-50 nM) resulted in 5-hydroxy[14C]tryptamine release comparable with that induced by with threshold doses of thrombin. However, ethanol did not inhibit phorbol ester-induced secretion. Ethanol also did not interfere with thrombin- or phorbol ester-induced phosphorylation of myosin light chain (20 kDa) or a 47 kDa protein, a known substrate for protein kinase C. By electron microscopy, ethanol had no effect on thrombin-induced shape change and pseudopod formation, but prevented granule centralization and fusion. The results indicate that ethanol does not inhibit platelet secretion by interfering with the activation of phosphoinositide-specific phospholipase C or protein kinase C by thrombin. Rather, the data demonstrate an inhibition of a Ca²⁺-mediated event such as granule centralization.

L26 ANSWER 12 OF 33 MEDLINE DUPLICATE 5
 ACCESSION NUMBER: 90141525 MEDLINE
 DOCUMENT NUMBER: 90141525 PubMed ID: 2300924
 TITLE: Human platelet factor V is crosslinked to actin by FXIIIA during platelet activation by thrombin.
 AUTHOR: Wang D L; Annamalai A E; Ghosh S; Gewirtz A M; Colman R W
 CORPORATE SOURCE: Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA 19140.
 CONTRACT NUMBER: CA 36896 (NCI)
 HL07248 (NHLBI)
 K04 CA01324 (NCI)
 SOURCE: THROMBOSIS RESEARCH, (1990 Jan 1) 57 (1) 39-57.
 Journal code: 0326377. ISSN: 0049-3848.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199003
 ENTRY DATE: Entered STN: 19900328
 Last Updated on STN: 19980206
 Entered Medline: 19900314

AB Although it has been established that factor V (FV) becomes associated irreversibly with the platelet cytoskeleton after stimulation with thrombin, the chemical nature of this complex is not known. Factor V has recently been demonstrated to be a substrate for factor XIIIa and to form factor V oligomers. We now show that thrombin-activated 125I-FV specifically links to a single protein (43 kDa) of the solubilized platelet membrane in a reaction which requires Ca⁺⁺ and factor XIIIa. In a **purified** system, FV, **activated** by **thrombin**, forms covalently linked high molecular complexes with 125I-actin catalyzed by factor XIIIa. The site of crosslinking of actin was the factor V fragments, 150 kDa (connecting peptide, C1) and its parent molecule 200 kDa (B). Using radiolabeled actin and unlabeled FV, factor XIIIa catalysed the formation of both homopolymers and heteropolymers. Unlabeled actin was found to compete with radiolabeled FV as a substrate for FXIIIa. To evaluate the biological significance of the crosslinking of factor V to actin, intact platelets were treated with B10 (monoclonal antibody to C1), or monospecific polyclonal antibodies to actin or FXIII. After stimulation with thrombin, the cytoskeleton (material insoluble in Triton X-100) showed markedly decreased 125I-FV in the crosslinked complexes. FV coagulant activity associated with platelet cytoskeleton was also diminished following incubation with an antibody to actin, factor XIII, or B10. These data suggest that FV, through the C1 domain, is crosslinked to actin in the cytoskeleton of thrombin-treated platelets. Activated factor XIII may play a role in plasma FV-platelet

interaction as well as the expression of FV derived from the alpha-granules on the cytoskeleton during platelet stimulation.

L26 ANSWER 13 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1988:2519 HCPLUS
DOCUMENT NUMBER: 108:2519
TITLE: Human factor VIII from heparinized plasma.
Purification and characterization of a single-chain form
AUTHOR(S): Ganz, Peter R.; Tackaberry, Eilleen S.; Palmer,
Douglas S.; Rock, Gail
CORPORATE SOURCE: Can. Red Cross, Ottawa Cent., Ottawa, ON, K1S 3E2,
Can.
SOURCE: European Journal of Biochemistry (1988), 170(3), 521-8
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human factor VIII was **purified** from human heparinized blood by cryopptn., PEG pptn., Affi-Gel blue, aminohexyl, polyelectrolyte E5, and immunoaffinity chromatog. A **purifn.** of 250,000-fold over plasma with a specific activity >5200 units/mg was achieved. Analyses of factor VIII by HPLC indicated a mol. mass of 28-340 kilodaltons (kDa). Variation in the native mass may reflect heterogeneity of the protein due to the assocd. lipid since structural anal. confirmed that factor VIII contained variable amts. of free fatty acids, diglycerides, and triglycerides, but no phospholipids. Addnl. characterization by denaturing PAGE under reducing conditions, followed by Ag staining, showed a major single-chain polypeptide of factor VIII with a mass of .apprx.260 kDa. To det. whether proteolyzed forms of factor VIII were present during fractionation, earlier steps in **purifn.** were analyzed. This revealed addnl. species of factor VIII eluting faster than the single-chain form during chromatog. on polyelectrolyte E5. Gel electrophoresis showed that these species of factor VIII consisted of multiple polypeptide chains, and partial peptide mapping with Staphylococcus aureus V8 protease indicated that they were structurally related. Monoclonal and hemophilic antibodies were used in immunoabsorption expts. to demonstrate that the **purified** factor VIII was composed predominantly of the 260-kDa factor VIII chain.

L26 ANSWER 14 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:5253 HCPLUS
DOCUMENT NUMBER: 110:5253
TITLE: The binding of **purified** factor VIII to platelets
AUTHOR(S): Ganz, Peter R.; Tackaberry, Eilleen S.; Rock,
Gail
CORPORATE SOURCE: Ottawa Cent., Can. Red Cross, Ottawa, ON, K1S 3E2,
Can.
SOURCE: Progress in Clinical and Biological Research (1988),
283(Platelet Membr. Recept.: Mol. Biol., Immunol.,
Biochem., Pathol.), 255-61
CODEN: PCBRD2; ISSN: 0361-7742
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Purified** factor VIII (I) bound specifically to **thrombin** -stimulated blood platelets, whereas the interaction with the unstimulated platelets apparently is nonspecific. Optimal binding to stimulated platelets occurred in 15-20 min of incubation, and this binding apparently is saturable at I concns. >12 mM. There were .apprx.104 mols. of I bound

per platelet at satn. Crosslinking studies suggested that I interacts closely with a platelet polypeptide of mol. wt. 80,000-90,000, as well as with other polypeptides (mol. wt. 240,000 or 1toreq.145,000). These mol. wts. are similar to those of platelet membrane glycoproteins GPV and GPIb.alpha., as well as to von Willebrand factor (vWF) and vWF:AgII, where Ag is antigen.

L26 ANSWER 15 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:108149 BIOSIS
DOCUMENT NUMBER: BR36:53565
TITLE: THE BINDING OF PURIFIED FACTOR VII TO PLATELETS.
AUTHOR(S): GANZ P R; TACKABERRY E S; ROCK G
CORPORATE SOURCE: OTTAWA CENTRE, CANADIAN RED CROSS, BLOOD TRANSFUSION SERVICE, DEP. BIOCHEMISTRY, UNIV. OTTAWA, OTTAWA, ONTARIO, CANADA K1S 3E2.
SOURCE: JAMIESON, G. A. (ED.). PROGRESS IN CLINICAL AND BIOLOGICAL RESEARCH, VOL. 283. PLATELET MEMBRANE RECEPTORS: MOLECULAR BIOLOGY, IMMUNOLOGY, BIOCHEMISTRY, AND PATHOLOGY; XIXTH ANNUAL SCIENTIFIC SYMPOSIUM OF THE AMERICAN RED CROSS, WASHINGTON, D.C., USA, OCTOBER 20-22, 1987. XVIII+727P. ALAN R. LISS, INC.: NEW YORK, NEW YORK, USA. ILLUS, (1988) 0 (0), 255-262.
CODEN: PCBRD2. ISSN: 0361-7742. ISBN: 0-8451-5133-9.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L26 ANSWER 16 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1987:47826 HCPLUS
DOCUMENT NUMBER: 106:47826
TITLE: Inhibition of protein cross-linking in calcium-enriched human erythrocytes and activated platelets
AUTHOR(S): Lorand, L.; Barnes, N.; Bruner-Lorand, J. A.; Hawkins, M.; Michalska, M.
CORPORATE SOURCE: Dep. Biochem., Mol. Biol. Cell Biol., Northwestern Univ., Evanston, IL, 60201, USA
SOURCE: Biochemistry (1987), 26(1), 308-13
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Treatment of human erythrocytes with Ca²⁺, in the presence of ionophore A 23187, caused the formation of high-mol.-wt. (>106) membrane protein polymers. This phenomenon, known to involve crosslinking of essentially all of the band 4.1 and 2.1 (ankyrin) proteins, as well as some spectrin, band 3, and Hb mols., could be prevented by preincubating the cells with a noncompetitive inhibitor of intrinsic transglutaminase, 2-[3-(diallylamino)propionyl]benzothiophene (I), at concns. of about (3-6) times. 10⁻⁴M. I also eliminated the proteolytic breakdown of the 2 major transmembrane proteins, band 3 and glycophorin, which would otherwise occur during the Ca²⁺ loading of fresh human red cells. In addn., I effectively blocked the formation of a crosslinked protein polymer in thrombin-activated human platelets.

L26 ANSWER 17 OF 33 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 85100403 MEDLINE
DOCUMENT NUMBER: 85100403 PubMed ID: 6518803
TITLE: A method for preparation of dry thrombin for topical application.
AUTHOR: Tsvetkov T; Bucureshtliev A; Alexiev N; Nicolov C;

SOURCE: Bucureshtlieva R; Mincheff M
CRYOBIOLOGY, (1984 Dec) 21 (6) 661-3.
Journal code: 0006252. ISSN: 0011-2240.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198502
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850226

AB Thrombin for topical hemostasis can be prepared from bovine or human blood plasma. The **prothrombin** is isolated by means of adsorption on DEAE-Sephadex A-50 and consecutively **activated** by **CaCl₂** and thromboplastin. **Thrombin** is precipitated and **purified** by acetone. The specific activity of the thrombin preparation is 122 + 23 IU/mg protein while the yield is 36,360 +/- 6623 IU/liter plasma.

L26 ANSWER 18 OF 33 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 83224380 MEDLINE
DOCUMENT NUMBER: 83224380 PubMed ID: 6407138
TITLE: **Stability** of VIII:C in plasma: the dependence on protease activity and calcium.
AUTHOR: Rock G A; Cruickshank W H; Tackaberry E S; Ganz P R; Palmer D S
SOURCE: THROMBOSIS.RESEARCH, (1983 Mar 1) 29 (5) 521-35.
Journal code: 0326377. ISSN: 0049-3848.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198307
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19830729

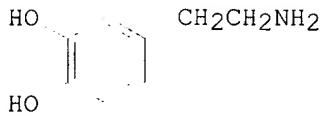
AB Loss of Factor VIII procoagulant activity (VIII:C) following blood collection is a major problem in providing sufficient amounts for therapeutic use and biochemical analyses. We have examined the effects of inhibition of plasma proteases and maintenance of physiological calcium ion on plasma VIII:C **stability**. The addition of protease inhibitors such as benzamidine, phenylmethylsulfonyl fluoride (PMSF), aprotinin, or soybean trypsin inhibitor (SBTI) to CPD plasma provided no significant protection against decay of VIII:C activity. Neither the rate of decay in the first 24 hours nor the final VIII:C activity observed after storage for 48-72 hours were significantly altered. On the other hand, addition of DFP or heparin to CPD plasma resulted in a marked improvement in VIII:C **stability** over 24 hours. This demonstrated that these two inhibitors are effective in preventing VIII:C degradation during storage. In addition to protease inhibition, the importance of maintaining physiological calcium ion was demonstrated by 100% **stabilization** of VIII:C in heparin plasma. Plasma obtained from CPD plus heparin blood could also be **stabilized** provided free calcium ion levels were restored to physiological concentrations. The inactivation of VIII:C in CPD plus heparin plasma was completely reversible up to 4 hours after collection. Studies on the recovery of activity after recalcification of CPD plus heparin plasma provided kinetic data which support a renaturation process of VIII:C rather than one due to enzymatic activation. The use of a **thrombin**-specific chromogenic

substrate revealed that after recalcification and during the recovery of VIII:C activity, there was no significant **thrombin** activity. Although the data suggest that proteolytic degradation plays some part in VIII:C decay, only the maintenance of physiologic calcium ion levels under cover of an effective non-chelating anticoagulant and protease inhibitor allows preservation of VIII:C activity.

L26 ANSWER 19 OF 33 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 83201931 MEDLINE
DOCUMENT NUMBER: 83201931 PubMed ID: 6405649
TITLE: The human blood platelet as a model for studying interactions of ethanol with membrane lipids.
AUTHOR: Fenn C G; Littleton J M
SOURCE: ALCOHOLISM, CLINICAL AND EXPERIMENTAL RESEARCH, (1983 Winter) 7 (1) 59-64.
Journal code: 7707242. ISSN: 0145-6008.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198306
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19980206
Entered Medline: 19830610

AB The effect of ethanol on human blood platelet aggregation is generally inhibitory, but aggregation caused by arachidonic acid is either unaffected or potentiated by ethanol. Of the other aggregatory agents tested, the **calcium** ionophore A23187, collagen, and **thrombin** were most inhibited by **ethanol**. These results suggest that in the case of collagen and ionophore A23187 ethanol may act to inhibit aggregation at some point between the rise in cytosolic calcium and the cleavage of membrane phospholipids associated with the platelet release reaction. A similar spectrum of inhibition was produced by the incorporation of unsaturated fats into the platelet or by reduction of divalent cations by addition of EDTA to the external medium. Platelets in which unsaturated fats were incorporated were less susceptible to inhibition by ethanol than those into which saturated fats had been incorporated.

L26 ANSWER 20 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1983:535310 HCPLUS
DOCUMENT NUMBER: 99:135310
TITLE: Comparison of effects of **ethanol** on platelet function and synaptic transmission
AUTHOR(S): Fenn, G. Christopher; Lynch, Marina A.; Nhamburo, Patson T.; Caberos, Louisa; Littleton, John M.
CORPORATE SOURCE: Dep. Pharmacol., King's Coll., London, WC2R 2LS, UK
SOURCE: Pharmacology, Biochemistry and Behavior (1983), 18(Suppl. 1), 37-43
DOCUMENT TYPE: Journal
LANGUAGE: English
GI



AB EtOH [64-17-5] inhibited human platelet (either platelet-rich plasma or gel-filtered platelets) aggregation by reducing the platelet release reaction. This action was due to inhibition of the activation by **Ca** of phospholipase A2 [9001-84-7], since A 23187 [52665-69-7]-induced release as well as release induced by collagen and **thrombin** [9002-04-4] were very much affected by EtOH; whereas arachidonic acid [506-32-1]-induced release was not inhibited by EtOH. A contrast to the inhibitory effects of EtOH on release of platelet contents in response to A 23187, the release of ³H-labeled dopamine (I) [51-61-6] from rat corpus striatum slices produced by 12 .mu.M A 23187 was enhanced by the presence of EtOH (50 mM) in the superfusing fluid. EtOH inhibited the activity of **Ca**-activated phospholipase A2 in rat synaptosomal prepns. Thus, although there are many similarities between the effects of EtOH on the platelet and synapse, there may be differences between the way in which EtOH modifies release of intracellular contents in the 2 situations.

L26 ANSWER 21 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 81130508 EMBASE
DOCUMENT NUMBER: 1981130508
TITLE: Isolation and partial characterisation of antiheparin proteins from avian thrombocytes.
AUTHOR: Wachowicz B.; Krajewski T.; Stefancyk B.
CORPORATE SOURCE: Dept. Biochem., Inst. Biochem. Biophys., Univ. Lodz, Poland
SOURCE: IRCS Medical Science, (1981) 9/4 (331-332).
CODEN: IRLCDZ
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
 025 Hematology
 018 Cardiovascular Diseases and Cardiovascular Surgery
LANGUAGE: English
AB Antiheparin proteins of avian thrombocytes have been **isolated** from **thrombin** released material and **purified** by **ethanol** precipitation technique and affinity chromatography. Heparin-Sepharose 4B chromatography has been applied to analyse the released proteins from duck and goose thrombocytes. Three protein fractions have been found. Most of the antiheparin activity was present in the last fractions eluted from the column with 1.5M NaCl. These fractions corresponded to pig platelet factor 4 (PF4) purified by a similar procedure. Whereas pig PF4 was homogeneous (Mr 13000) avian thrombocyte fractions analysed by SDS-polyacrylamide gel electrophoresis revealed heterogeneity. Moreover, the specific antiheparin activity of pig platelets was 2-3 times greater than of avian thrombocytes.
L26 ANSWER 22 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1980:21854 BIOSIS
DOCUMENT NUMBER: BR18:21854
TITLE: ISOLATION OF ANTI THROMBIN III WITHOUT INTERFERING WITH ETHANOL FRACTIONATION SYSTEM.
AUTHOR(S): WICKERHAUSER M; WILLIAMS C

CORPORATE SOURCE: AM. RED CROSS BLOOD SERV. LAB., BETHESDA, MD., USA.
SOURCE: 7TH INTERNATIONAL CONGRESS ON THROMBOSIS AND HAEMOSTASIS,
LONDON, ENGLAND, JULY 15-20, 1979. THROMB HAEMOSTASIS,
(1979) 42 (1), 168.
CODEN: THHADQ. ISSN: 0340-6245.

DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L26 ANSWER 23 OF 33 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 9
ACCESSION NUMBER: 1977:598140 HCAPLUS
DOCUMENT NUMBER: 87:198140
TITLE: Some effects of calcium on the activation of human factor VIII/von Willebrand factor protein by thrombin
AUTHOR(S): Switzer, Mary Ellen; McKee, Patrick A.
CORPORATE SOURCE: Dep. Med., Duke Univ. Med. Cent., Durham, NC, USA
SOURCE: Journal of Clinical Investigation (1977), 60(4), 819-28
CODEN: JCINAO; ISSN: 0021-9738
DOCUMENT TYPE: Journal
LANGUAGE: English
AB When Factor VIII (I) was chromatographed on 4% agarose in 0.25M CaCl₂, the protein and von Willebrand factor (vWF) activity appeared in the void vol., but most of the I procoagulant activity eluted later. Delayed I procoagulant activity may be a proteolytically modified form of I/vWF protein. To test if thrombin is the protease involved, the effect of 0.25M CaCl₂ on I/vWF and its reaction with thrombin was exampd. About 30% of the I procoagulant activity was lost immediately when solns. were made 0.25M in CaCl₂. When I in 0.15M NaCl was activated with thrombin and then made 0.25M in CaCl₂, the procoagulant activity of a broad range of I/vWF protein concns. remained activated for >treq.6 h. But, in 0.25M CaCl₂, the increase in I procoagulant activity in response to thrombin was much more gradual and once activated, the procoagulant activity was stabilized by 0.25M CaCl₂. When thrombin-activated I/vWF protein was filtered on 4% agarose in 0.15M NaCl, there was considerable inactivation of I procoagulant activity. When thrombin-activated I/vWF protein was filtered in 0.25M CaCl₂, the I procoagulant activity eluted well after the void vol. and remained activated for 6 h. The procoagulant peak isolated by filtering nonthrombin-activated I/vWF protein on agarose in 0.25M CaCl₂ was compared to that isolated from thrombin-activated I/vWF protein. Both procoagulant activity peak proteins had about the same sp. vWF activity as the corresponding void-vol. protein. Before redn., gel patterns for the 2 procoagulant activity peak proteins were the same. After redn., the gel pattern for the nonthrombin-activated procoagulant activity peak protein contained bands of 195,000, 148,000-120,000, 79,000, 61,000, 51,000, and 18,000 daltons, whereas the pattern for the reduced thrombin-activated procoagulant activity peak protein lacked the higher mol. wt. bands, but consistently showed the 4 lower mol. wt. bands. Thrombin appears to generate the I procoagulant activity that is stabilized by 0.25M CaCl₂ and elutes aberrantly from 4% agarose.

L26 ANSWER 24 OF 33 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 76062220 MEDLINE
DOCUMENT NUMBER: 76062220 PubMed ID: 1201228
TITLE: Alternative pathways for the activation of factor XIII.
AUTHOR: McDonagh J; McDonagh R P
SOURCE: BRITISH JOURNAL OF HAEMATOLOGY, (1975 Aug) 30 (4) 465-77.
Journal code: 0372544. ISSN: 0007-1048.

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197603
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 20000303
Entered Medline: 19760301

AB Factor XIII is present in plasma as a proenzyme, which when activated catalyses the formation of epsilon(gamma-glutamyl)lysyl bonds in fibrin. In this study the activation of purified plasma factor XIII was examined quantitatively with the fluorescent amine incorporation assay. Activation products were examined by polyacrylamide gel electrophoresis. The serin proteases, thrombin, trypsin, chymotrypsin, and factor Xa, and also Reptilase were tested for their ability to activate factor XIII. Highly purified thrombins activated purified factor XIII; this reaction was not calcium dependent. Trypsin was also a potent activator, but no transglutaminase activity was found with chymotrypsin. The most highly purified preparations of Reptilase had no effect on factor XIII activity. Less purified Reptilase preparations activated factor XIII, which suggests the presence of another enzyme in these Reptilase preparations. Highly purified factor Xa was found to be an effective **activator** of **purified** factor XIII. In contrast to **thrombin activation**, this reaction required **calcium**. It may be that under certain circumstances factor XIIIa could be formed in vivo directly by the alternative pathway of factor Xa. Factor XIIIa could then crosslink fibrinogen, which would also provide an alternative pathway for thrombus formation. Also, the activation of factor XIII by both factor Xa and thrombin provides a further point of control in the blood coagulation process.

L26 ANSWER 25 OF 33 MEDLINE
ACCESSION NUMBER: 2001241227 MEDLINE
DOCUMENT NUMBER: 21241823 PubMed ID: 11344575
TITLE: Fibrinogen Philadelphia. A hereditary hypofibrinogenemia characterized by fibrinogen hypercatabolism.
AUTHOR: Martinez J; Holburn R R; Shapiro S S; Erslev A J
CORPORATE SOURCE: Cardeza Foundation for Hematologic Research and Departments of Medicine and Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA.
CONTRACT NUMBER: HE-09163 (NHLBI)
HE-14566
HL-06374
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1974 Feb) 53 (2)
600-11.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719

AB A new, autosomally inherited abnormal fibrinogen associated with hypofibrinogenemia has been described in several members of a family. Plasma fibrinogen measured either as thrombin-clottable protein or by immunodiffusion revealed a fibrinogen level ranging between 60 and 90

mg/100 ml. The thrombin time of plasma or purified fibrinogen was prolonged and only partially corrected by the addition of **calcium**. **Purified** fibrinogen prolonged the **thrombin** time of normal plasma. Fibrinopeptide release by thrombin was normal in rate and amount, but fibrin monomer aggregation was grossly disturbed, especially in a high ionic strength medium. We have designated this fibrinogen "fibrinogen Philadelphia." Acrylamide gel electrophoresis of mixtures of [121I]normal and [125I]abnormal fibrinogens revealed a slight increase in the anodal mobility of fibrinogen Philadelphia. Similarly, DEAE-cellulose chromatography showed slightly stronger binding of fibrinogen Philadelphia than normal. To elucidate the mechanism responsible for the low plasma fibrinogen concentration, simultaneous metabolic studies of autologous (patient) and homologous (normal) fibrinogen, labeled with 125I and 121I, respectively, were performed in two affected subjects. Autologous fibrinogen half-life was short and the fractional catabolic rate was markedly increased in both family members. In contrast, homologous fibrinogen half-life and fractional catabolic rate were normal. These metabolic studies demonstrate that rapid degradation of fibrinogen Philadelphia is largely responsible for the depressed levels of a plasma fibrinogen. This represents the first example of a mutant plasma protein in which the molecular defect is associated with an altered catabolism.

L26 ANSWER 26 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 75066862 EMBASE

DOCUMENT NUMBER: 1975066862

TITLE: The **calcium** induced dissociation of human **plasma** clotting factor XIII.

AUTHOR: Cooke R.D.; Holbrook J.J.

CORPORATE SOURCE: Dept. Biochem., Univ. Bristol, United Kingdom

SOURCE: Biochemical Journal, (1974) 141/1 (79-84).

CODEN: BIJOAK

DOCUMENT TYPE: Journal

FILE SEGMENT: 029 Clinical Biochemistry

025 Hematology

LANGUAGE: English

AB Large quantities of human Factor XIII were prepared from **ethanol** precipitates of outdated human **plasma**. Material homogeneous after chromatography on DEAE cellulose was further resolved into 2 proteins, A and B, after **filtration** on Sepharose 6B. Protein A has a molecular weight of 350000 and a subunit structure a2b2 and is activated by **thrombin** and **calcium**. Protein B is inactive and probably has a subunit structure b2. **Calcium** causes protein A, after **thrombin** cleavage, to fragment to give protein B and a protein, containing only a' subunits, which is catalytically active. The latter protein slowly forms a misty precipitate which is still active and not cross linked covalently. This confirms the suggestion of Schwartz et al. that catalytic activity is only associated with a' subunits. Iodoacetate, which inhibits the enzyme, does not inhibit dissociation and aggregation of protein A. The existence of 2 proteins and the fragmentation are possible explanations for the wide range of molecular weights given for Factor XIII in the literature.

L26 ANSWER 27 OF 33 HCPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1964:69364 HCPLUS

DOCUMENT NUMBER: 60:69364

ORIGINAL REFERENCE NO.: 60:12264g-h

TITLE: Separation of autoprothrombin III from bovine **prothrombin** preparations

AUTHOR(S): Seegers, Walter H.; Cole, Edmond R.; Aoki, Nobuo;

Harmison, Charles R.
CORPORATE SOURCE: Wayne State Univ., Coll. of Med., Detroit, MI
SOURCE: Can. J. Biochem. (1964), 42(2), 229-33
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB Purified **prothrombin** was activated by means of **purified thrombin**, Ac-globulin, **Ca** ions, and crude cephalin. Autoprothrombin III was isolated as a single component by the same methods found suitable for the isolation of autoprothrombin C. It contained no autoprothrombin C activity, but some was generated spontaneously. It may be that autoprothrombin C generally does not form in normal blood clotting unless tissue exts. are involved. This implies the possibility that the most potent procoagulant power in the genesis of thrombosis is derived from tissues.

L26 ANSWER 28 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1962:424779 HCPLUS
DOCUMENT NUMBER: 57:24779
ORIGINAL REFERENCE NO.: 57:5009h-i,5010a-b
TITLE: Autoprothrombin C: a second enzyme from **prothrombin**
AUTHOR(S): Marciniak, Ewa; Seegers, Walter H.
CORPORATE SOURCE: Wayne State Univ., Detroit, MI
SOURCE: Can. J. Biochem. Physiol. (1962), 40, 597-605
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB In addn. to thrombin, there is another deriv. of **prothrombin** which is an end product of **prothrombin** activation. It is an accelerator of **prothrombin** activation, and is called autoprothrombin C (I). The activity develops from purified bovine **prothrombin** in 25% Na citrate soln. simultaneously with thrombin. I was sepd. from thrombin by chromatography on Amberlite IRC-50. The fraction which seps. from thrombin has esterase activity and very likely this esterase activity is assocd. with the I mol. Since the I and the thrombin are both derived from **prothrombin**, at least 2 enzymes are the end products of **prothrombin** activation. I catalyzed the activation of purified **prothrombin** in 25% Na citrate soln., and this function was easily inhibited with p-toluenesulfonyl-L-arginine methyl ester. I preps. were mixed with platelets, Ac-globulin, and **Ca⁺⁺** to obtain rapid conversion of **purified prothrombin** to **thrombin**. This activation mixt. did not generate I and some unspecified substance most likely needs to be added in order to obtain the I activity. The activity developed together with thrombin when tissue exts., Ac-globulin, and **Ca⁺⁺** were used for the activation of **prothrombin**. I is relatively stable over the pH range 5.5 to 8.5. It is stable up to 56.degree. for 30 min. Plasma contains a substance that inactivates I.

L26 ANSWER 29 OF 33 HCPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1956:90813 HCPLUS
DOCUMENT NUMBER: 50:90813
ORIGINAL REFERENCE NO.: 50:17092i,17093a
TITLE: Quantitative concepts related to **prothrombin** and autoprothrombin I activity
AUTHOR(S): Seegers, Walter H.; Johnson, Shirley A.; Penner, John A.
CORPORATE SOURCE: Wayne Univ., Detroit, MI
SOURCE: Can. J. Biochem. and Physiol. (1956), 34, 887-92
DOCUMENT TYPE: Journal

- LANGUAGE: Unavailable
AB Russell viper venom can be combined with autoprothrombin I and calcium ions for the activation of purified prothrombin to thrombin provided a small amt. of Ac-globulin is also in the reaction mixt. This fact is utilized for developing a quant. assay for autoprothrombin I. It is estd. that the autoprothrombin I concn. of plasma is from 1 to 1.5 mg. %.
- L26 ANSWER 30 OF 33 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1957:6106 HCAPLUS
DOCUMENT NUMBER: 51:6106
ORIGINAL REFERENCE NO.: 51:1354f-g
TITLE: Calcium-ion requirement for threone activity
AUTHOR(S): Johnson, Shirley A.; Seegers, Walter H.
CORPORATE SOURCE: Wayne Univ., Detroit, MI
SOURCE: Proc. Soc. Exptl. Biol. Med. (1956), 92, 597-8
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB When threone (cf. C.A. 48, 11521b) is involved in the conversion of purified prothrombin to thrombin, the optimal Ca concn. ranges from 0.009M to 0.04M. This is a much broader range than found when thromboplastin is used for the activation of prothrombin.
- L26 ANSWER 31 OF 33 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1954:64643 HCAPLUS
DOCUMENT NUMBER: 48:64643
ORIGINAL REFERENCE NO.: 48:11520i,11521a-b
TITLE: Relationship of certain antihistamine drugs to the activation of purified prothrombin
AUTHOR(S): Murray, Marvin; Johnson, Shirley A.; Seegers, Walter H.
CORPORATE SOURCE: Wayne Univ., Detroit, MI
SOURCE: Am. J. Physiol. (1954), 178, 10-16
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. preceding abstr. Purified prothrombin transforms to thrombin in solns. with Ca, small amts. of thrombin, platelet exts., and linadryl. Histamine, benadryl, decapryl, and phenindamine may be used in place of linadryl. It is suggested that these org. compds. substitute for platelet cofactor I (anti-hemolytic factor) in the activation of prothrombin under the conditions of the expts. It is proposed that the word threone be used to refer to the activity characteristic of the combinations of platelets and linadryl, platelets and any of the other active compds., or platelet exts. and plasma cofactor I. It is postulated that thromboplastin can act directly with plasma Ac-globulin, whereas threone is dependent upon the transformation of plasma Ac-globulin to serum Ac-globulin by small amts. of thrombin.
- L26 ANSWER 32 OF 33 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1948:32224 HCAPLUS
DOCUMENT NUMBER: 42:32224
ORIGINAL REFERENCE NO.: 42:6867g-i
TITLE: Studies on prothrombin: purification, inactivation with thrombin, and activation with thromboplastin and calcium
AUTHOR(S): Ware, Arnold G.; Seegers, Walter H.

CORPORATE SOURCE: Coll. Med., Wayne Univ., Detroit, MI
SOURCE: J. Biol. Chem. (1948), 174, 565-75
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB cf. C.A. 42, 3056i. **Prothrombin** was prep'd. as previously described. The small amt. of Ac-globulin remaining was destroyed by heating the **prothrombin** to 53.degree. in neutral distd. water for 2 hrs. Small amts. of thrombin (1 unit) cause large amts. of **prothrombin** (10,000 units) to be unstable. Larger amts. of thrombin first destroy the **prothrombin** and then cause a regeneration of **prothrombin** with somewhat different properties. In the absence of Ac-globulin the activation of the **prothrombin** by Ca ions and thromboplastin is slow.

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DOCUMENT NUMBER: 38:18811
ORIGINAL REFERENCE NO.: 38:2712h-i,2713a
TITLE: Blood thrombokinase
AUTHOR(S): Reichel, Christian
SOURCE: Klin. Wochschr. (1942), 21, 1081-2
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB A purified **prothrombin**-thrombokinase soln. was obtained by pptn. of euglobulin from dild. human serum. Highly active thrombin could be prep'd. by incubation with Ca ions. Thus, it is shown that blood thrombokinase occurs as an inactive precursor in the serum proteins and can be mobilized from them. It must be assumed that analogous conditions occur within the vessels. In contrast to normal plasma and blood coagulation, upon **thrombin** formation in the **purified** system, **prothrombin**-thrombokinase-Ca ions, the total amt. of **prothrombin** is converted into thrombin.